UDC 582.736.312 : 664.642(045)
DOI: 10.18372/2306-1472.74.12303

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EFFECT OF ALFALFA MEDICAGO SATIVA ON VITAL ACTIVITY OF YEAST S. CEREVISIAE

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Purpose: *improvement of biotechnological qualities of baker's yeast promotes the intensification of fermentation processes, semis maturation, and ultimately the improvement of the quality of final product. Yeasts do not always satisfy the requirements. Therefore studies that are aimed at the intensification of fermentative activity and improvement of the quality of yeasts are interest. The problem of examination of stimulating effect of substances from different nature and origin on the growth and quality of yeasts is not new. One of the methods of intensification of the technological process of yeast production is selection of culture medium by replacement of mineral source of nitrogen with plant-based source. In our opinion, it is suitable to use for this purpose.* ***Methods:*** *in this study, we examined the ability of yeast cells to utilize organic nitrogen from a plant-based source of protein such as alfalfa granules. The use of this method allowed us to carve out the optimal components of the nutrient medium, which has provided the yeast with improved quality indicators.* ***Results****: we examined the effect of alfalfa as a source of nitrogen on the vital activity of yeast S. cerevisiae.* ***Discussion****: Alfalfa granules can be used in a cultural medium for barm yeast cultivation on a production scale.*

Keywords: alfalfa; biomass; culture medium; nitrogen source; *yeast S. cerevisiae.*

1. Introduction

Baker's yeast is used in various areas of industry: food industry (including bread baking), medical and microbiological industries. It is also used in confectionery, canning and other manufactures [1].

Yeast is cultivated on sugar-containing environments with provision of nitrogenic and phosphoric nutrition in the presence of dissolved oxygen. *Saccharamyces cerevisiae* yeast digests only two forms of nitrogen: ammonium salts and organic compounds. These microorganisms effectively utilize ammonium sulfate, carbamide, ammonia salts of acetic, lactic, malic and succinic acid, metal ammine complexes, amino acids, heterocyclic compounds. For yeasts, ammonia salts are only a source of nitrogen, but when nitrogen is used, acids that change pH of the solution are freed. Nitrogen from ammonia is utilized by yeasts better than other amino acids [2].

Amino acids are simultaneously a source of nitrogen and carbon, wherein the latter is assimilated from keto acids that are formed as a result of amino groups splitting off. Direct assimilation of amino acids from culture medium that contains full set of amino acids and any carbohydrates that are fermented is also possible. Because of the assimilation of amino acids, protein synthesis is provided, including enzymes that accelerate the process of yeast cells budding. As a result, the sugar intake of the medium for the nutrition of the yeast are lowered.

To expense organic nitrogen (amino acids, amides) many yeasts require vitamins (biotin, panthothenic acid, thiamine, pyridoxine etc.). Yeasts do not utilize such nitrogen compounds as proteins, betaine, choline, purines, amines and ethyl-, propylamine [2].

Peptides occupy middle position between amino acids and proteins. Yeast consumption of peptides is

lowered with the increase of their complexity. Some amount of peptides in the medium close to other forms of nitrogen contributes to the amino acid utilization. Carbamide, which is utilized by the yeasts without formation of acid radical and altering of pH of the solution, is considered to be the best source of nitrogen nutrition.

2. Analysis of recent research and publications, problem statement

Essential disadvantage of mineral sources of nitrogen is that they have a certain class of danger, therefore storage of such materials is problematic and to a certain extent threatens environmental friendliness of the enterprise. This problem creates interest that causes the search for other sources of nitrogen nutrition of yeasts, that have ecological significance for the enterprise [5].

Alfalfa is a valuable high protein perennial fodder crop, which is widely used in fodder production in many countries. It occupies the first place from fodder crops by the value of forage mass: 100 kg of hay contains up to 14 kg digestible protein [3,4]. Moreover, alfalfa is rich in vitamins and mineral substances (vitamins A, D, E, K, C, B_1, B_2, B_6, niacin, panthothenic acid, inositol, biotin and folic acid; minerals: phosphorus, calcium, potassium, sodium, chlorine, sulfur, magnesium, copper, manganese, iron, cobalt, boron, molybdenum; microelements: nickel, lead, strontium and palladium) [6-10].

3. Purpose of the study

The purpose of the study was the examination of effect of features of the yeast metabolism in the conditions of bringing in additional nitrogen nutrition on the indicators of yeast's vital activity.

The first stage of the experiment was the selection of clear culture of the yeast for subculturing onto a liquid culture medium for gradual accumulation. 0.1 g of baker's pressed yeast was sterilely brought in to two test-tubes containing 10 cm^3 of malt wort with 8-10% concentration of dry substances and was cultivated in a thermostat at 28^{o}C temperature during 48 hours. After that the obtained cultural liquid was transferred from each test-tube into a flask with 200 cm^3 of sterile malt wort.

One of the flasks was used as control and mineral salts of ammonium and phosphorus were additionally put into it (10% of the volume of the culture medium). Granules of alfalfa as a source of nitrogen in terms of the necessary amount for optimal growth of the yeast were put into the other flask. Both flasks were put in a thermostat at 28 ^{o}C for 72 hours.

Every 6 hours samples for the determination of the indicators of yeast's vital activity were taken, specifically:

- pH;
- Titratable acidity;
- Cell count;
- Glycogen content;
- Dead cell count.

Cell counting was done using hemocytometer with standard method and was calculated by the following formula:

$$?=\left(wa\frac{4000b}{c}\right)100 \qquad (1)$$

Indicators of active acidity were measured using a pH-meter. Values of titratable acidity were measured using titration cultural liquid with 0,1n NaOH solution in the presence of indicator phenolphthalein and volume of sodium hydroxide, that was spent on neutralization of present acids in the studied liquid, was recorded. Determination of dead cell count and glycogen content was done using microscopic method. A drop of cultural liquid was put on a microscope slide and a drop of methylene blue dye was added to count dead cells. Drops were mixed and held for 2-3 minutes, then were covered with cover glass and microscoped with x400 magnification.

Initially all cells were counted, then only dyed (dead) cells. Counting was conducted in five different microscopic field and dead cell count was calculated as a percentage.

Glycogen is a storing nutritional substance which appears in yeast after the moment of fermentation of 2/3 of sugars in wort. A drop of unfiltered yeast and 2-3 drops of 0.5% iodine solution were put on a microscope slide to determine glycogen. Drops are mixed, covered with cover glass, excess liquid is removed using filter paper. After 2-3 minutes, cytoplasm of yeast cells is dyed in light yellow color and glycogen granules are dyed in brick-red. In normal yeast glycogen takes from 1/3 to 2/3 of cell volume. If glycogen takes less than 1/4 of cell volume, its amout is considered insufficient. Young yeast is dyed in light yellow color by iodine. Glycogen is absent in old or hungry cells.

4. Results and discussion.

It was shown that cell count grows in the culture medium that contains alfalfa compared to control (Fig. 1). Cell count in 1 cm^3 was 102.3 mln at 48 hours, and 151.4 mln of cells at 72 hours, which is higher than in standard cultural medium by 11.7% and 12.2% respectively. It can be related to the fact that the chemical composition of alfalfa contains a significant amount of substances which are necessary for yeast growth and reproduction, and contributes to their rational digestion.

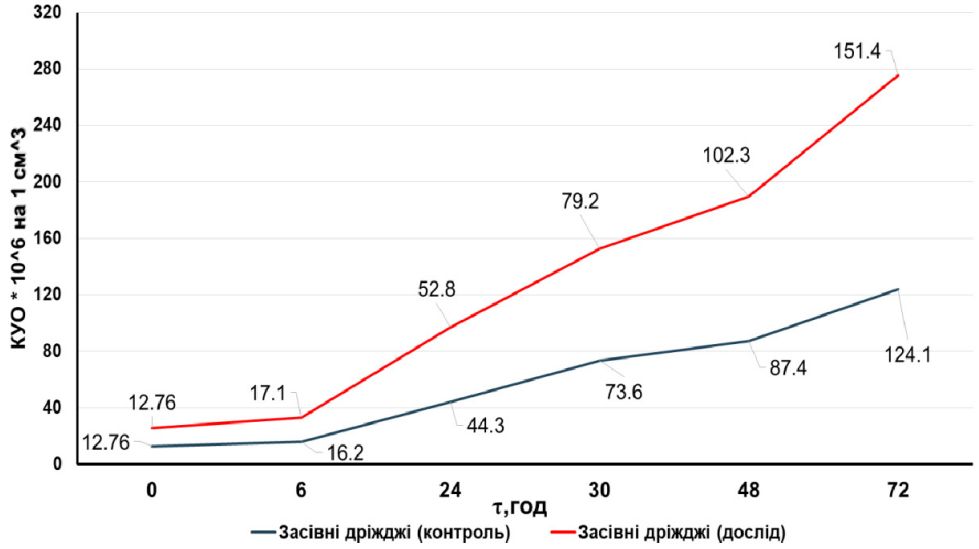


Fig. 1. Yeast cell count growth dynamics

After determining acidity indicators for both cultural mediums a conclusion can be made that they do not differ from one another significantly. Therefore, it shows that the medium containing alfalfa does not impair growth and development of yeast.

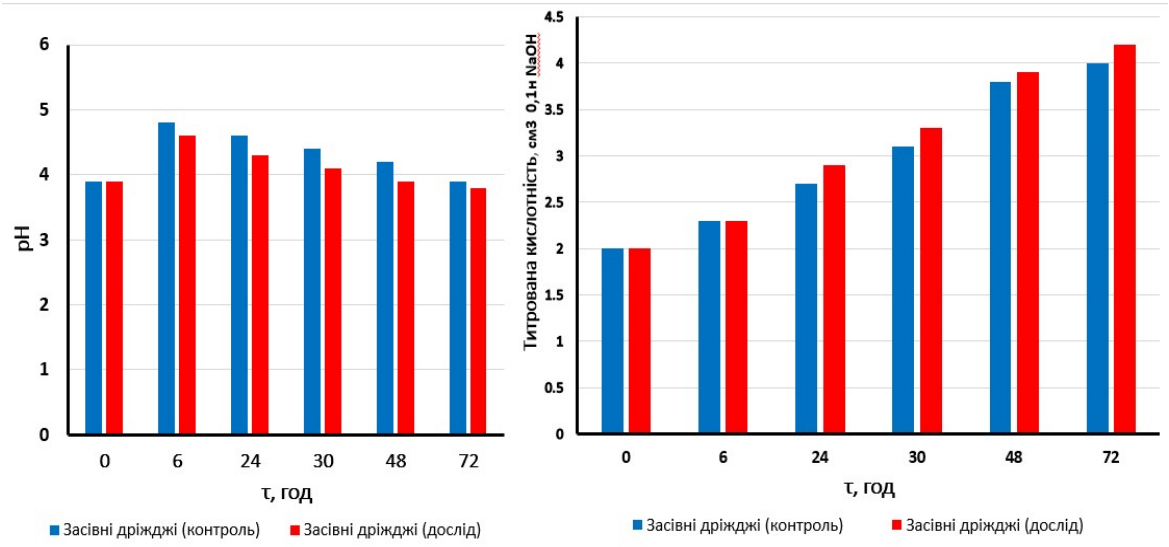


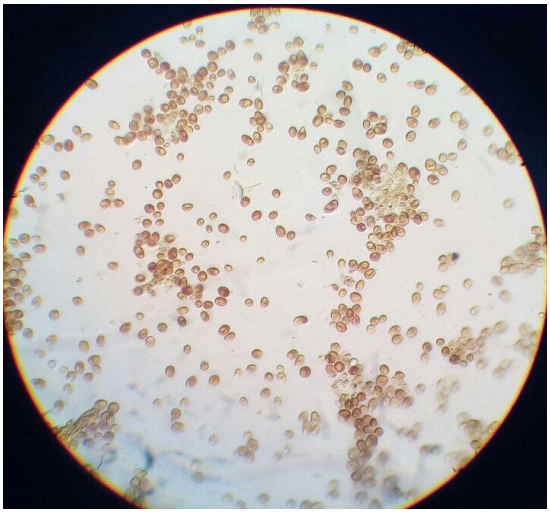
Fig. 2. Change of active and acidity in yeast cultivation

Polysaccharide content, specifically glycogen is determined with a goal of evaluation of effect of substratum on formation of reserve substances in the cells of the microorganisms in the obtained biomass.

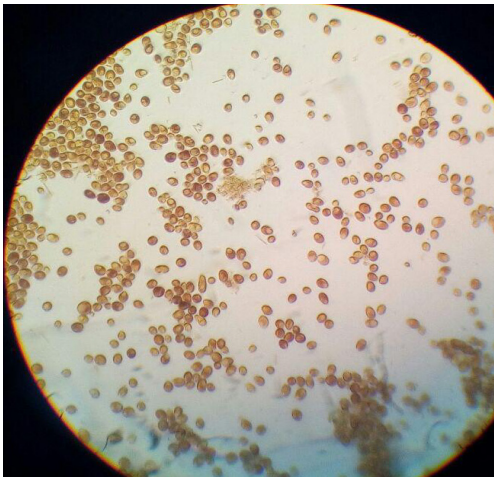
It was shown that the yeast *Saccharomyces cerevisiae* which was cultured in the medium with alfalfa had more glycogen at days 2 and 3 than the yeast that was cultured in a conventional cultural medium (Fig. 3). Intensity of coloration that corresponds to reserve carbohydrate content is shown on the pictures below.

At the 48th hour of cultivation (Fig. 3 a, b) both samples have shown sufficient for matured yeast glycogen content, but at the 72nd hour carbohydrate content in yeast in the control was significantly lowered on account of it's utilization at a deficit of carbon in the cultural medium. On the contrary, glycogen content in the medium containing alfalfa remained unaltered.

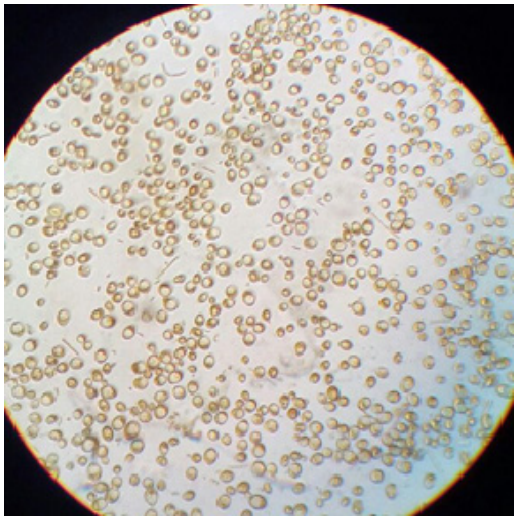
From the obtained data an assumption can be made which points out that alfalfa contributes to reserve carbohydrate accumulation, because of the fact that in protein decomposition amino acids are formed, which in turn are decomposed to amino and carbon acids, so an additional nutritional source for yeast cells emerges.



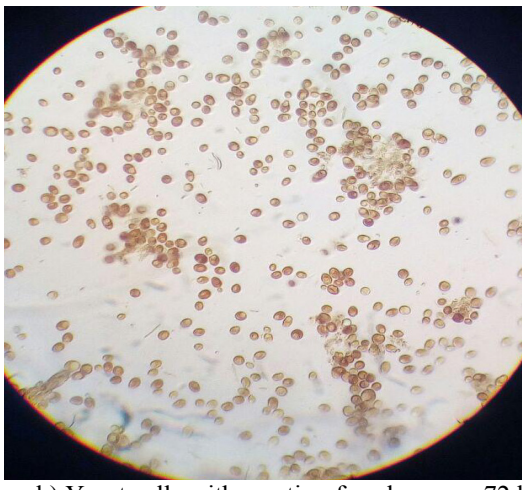
a) Yeast cells with reaction for glycogen, 48 hours (control)



b) Yeast cells with reaction for glycogen, 48 hours (experiment)



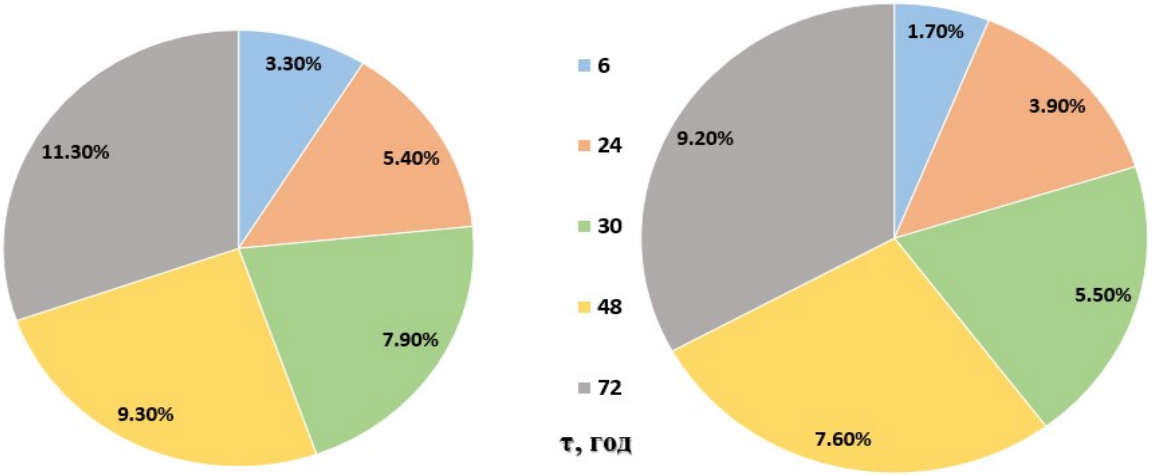
a) Yeast cells with reaction for glycogen, 72 hours (control)



b) Yeast cells with reaction for glycogen, 72 hours (experiment)

Fig. 3. Picture of yeasts with reaction for glycogen content using x400 magnification

It was found that the dead cell count (Fig. 4) in cultural medium containing alfalfa is lower than in the control cultural medium at all stages of cultivation. I.e. alfalfa contributes to yeast cells reproduction, which unequivocally has a positive effect for further application at a production scale. In our opinion this result is related to a sufficient glycogen content even at the 72nd hour of yeast maturation, which gives the cells an opportunity to rationally utilize all the necessary resources and to maintain maximal duration of the life cycle.



a) Barm yeast (control) b) Barm yeast (experiment)

Fig 4 (a, b) Change of dead cell count on different stages of yeast cultivation

5. Conclusions

Established the growth of biomass on a nutrient medium with alfalfa was 12.2% more than in control however, the number of dead cells was 2.1% less. It is shown that alfalfa Medicaqo sativa, as a source of nitrogen, has a positive effect on the growth and development of yeast. This implies that this plant has the advantage of over mineral sources of nitrogen and to improve the sustainability of production.

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Received 15 December 2017

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Вплив люцерни посівної *Meticago sativa* на життєдіяльність дріжджів *Saccharomyces cerevisiae*

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Мета: удосконалення біотехнологічних властивостей хлібопекарських дріжджів сприяє інтенсифікації процесів бродіння, дозрівання напівфабрикатів і, в остаточному підсумку, поліпшення якості готової продукції. Дріжджі не завжди задовольняють пропонованим до них вимогам. Тому дослідження спрямовані на інтенсифікацію бродильної активності і підвищення якості дріжджів є актуальними. Проблема дослідження стимулюючої дії речовин різної природи і походження на розвиток і якість дріжджів не є новою. Один із способів інтенсифікації технологічного процесу виробництва дріжджів - підбір поживного середовища за рахунок заміни мінерального джерела азоту на рослинне. **Методи**: у цій роботі була досліджена здатність дріжджових клітин споживати органічний азот з рослинного джерела білку, в якості гранул люцерни. Застосування цього методу дозволило виділити оптимальні компоненти живильного середовища, що забезпечило дріжджам поліпшені показники якості. **Результати**: досліджено вплив люцерни в якості джерела азоту на життєдіяльність дріжджів *S. cerevisiae*. **Обговорення**: гранули люцерни можна використовувати в поживному середовищі для культивування засівних дріжджів у виробничих масштабах.

Ключові слова: біомаса; джерело азоту; дріжджі *S. cerevisiae;* люцерна; поживне середовище.

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Влияние люцерны посевной *Meticago sativa* на жизнедеятельность дрожжей *Saccharomyces cerevisiae*

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Цель: улучшение биотехнологических свойств хлебопекарных дрожжей способствует интенсификации процессов брожения, созревания полуфабрикатов и, в конечном итоге, улучшению качества готовой продукции. Дрожжи не всегда удовлетворяют предъявляемым к ним требованиям. Поэтому исследования, направленные на интенсификацию бродильной активности и повышение качества дрожжей, являются актуальными. Проблема исследования стимулирующего действия веществ различной природы и происхождения на развитие и качество дрожжей не является новой. Один из способов интенсификации технологического процесса производства дрожжей - подбор питательной среды за счет замены минерального источника азота на растительное**. Методы:** в данной работе была исследована способность дрожжевых клеток потреблять органический азот из растительного источника белка, в качестве гранул люцерны. Применение этого метода позволило выделить оптимальные компоненты питательной среды, что обеспечило дрожжам улучшенные качественные показатели. **Результаты:** изучено влияние люцерны как источника азота на жизнедеятельность дрожжей *S. cerevisiae*. **Обсуждение:** гранулы люцерны можно использовать в питательной среде для культивирования засевных дрожжей в производственных масштабах.

Ключевые слова: биомасса; дрожжи *S. cerevisiae;* источник азота;люцерна; питательная среда.

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